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EPR Study of Cation Radicals Derived from Benz[*a*]anthracene and Its Monomethylated Derivatives on Oxidation with Thallium(III) Trifluoroacetate

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EPR spectra of cation radicals from benz[*a*]anthracene (BA), 12 monomethylated BAs, and 7,12-dimethyl-BA have been observed upon oxidation with thallium tristrifluoroacetate in trifluoroacetic acid (TTFA/TFA). The species giving rise to the EPR spectrum in BA/TTFA/TFA was determined to be the 7-trifluoroacetoxy-BA cation radical and its EPR spectrum has been analyzed by numerical methods in terms of 11 nonequivalent proton splittings and 3 equivalent fluorine splittings. An assignment of the splitting constants has been proposed by comparison with the EPR spectra of the 12 monomethylated BA cation radicals. The spin density distribution in the HOMO of BA has thus been experimentally determined.

Benz[*a*]anthracene (BA) (Fig. 1) is a weakly carcinogenic molecule (*1*), substitution of a methyl group at the 7, 12, 6, and 8 positions leads to substantially enhanced carcinogenicity (*2, 3*), and 7,12-dimethylbenz[*a*]anthracene (DMBA) is among the strongest carcinogenic polycyclic hydrocarbons (*1*). The role of cation radical intermediates in the activation of these molecules to ultimate carcinogens has not been firmly established, although much circumstantial evidence suggests that these molecules may undergo a biological one-electron oxidation (*4, 5*). Few EPR studies of BA cation radicals have been reported. Elmore and Forman (*6*) in 1976 published spectra from BA and DMBA in H_2SO_4, which were poorly resolved due no doubt to the nonequivalence of all the splittings in the molecule. In several previous papers (*7–10*) methods have been outlined which have enabled the EPR spectra of the equally complex benzo[*a*]pyrene cation radicals to be analyzed. In this work some of these methods have been applied to the cation radicals derived from BA and its methylated derivatives on oxidation with thallium(III) trifluoroacetate (TTFA) in trifluoroacetic acid (TFA). In addition a method of data analysis of EPR spectra using the SIMPLEX optimization method (*11*) has been developed. This method differs somewhat from the only previous application of the SIMPLEX method in EPR spectroscopy (*12*).

EXPERIMENTAL

Samples of 3 mg of 1- through 12-monomethylbenz[*a*]anthracenes (ME-BAs) were obtained from the National Cancer Institute Chemical Repository. BA and

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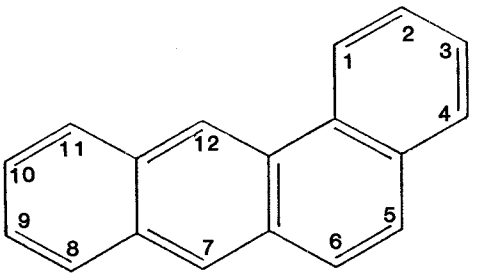

FIG. 1. Structure of benz[a]anthracene.

DMBA were commercially available samples of highest purity (Eastman Kodak). 7-d-BA was kindly supplied by Dr. B. Rickborn (*13*). Oxidation of BA and derivatives was carried out by dissolving 0.1–0.5 mg of the appropriate hydrocarbon in degassed TFA, followed by addition of a few drops of 0.8 *M* TTFA in TFA. The mixture was then degassed under vacuum using several freeze–pump–thaw cycles. The sample was allowed to warm to room temperature for a few minutes to allow reaction to occur before it was placed in the cooled EPR cavity. EPR spectra were typically run at about −15°C on a Varian E-9 spectrometer.

For data analysis an Apple IIe microcomputer was interfaced to the EPR spectrometer with an ADALAB board, to which the output voltage of the spectrometer is directly sent. A data acquisition program collects a maximum 4K data points during a user-specified scan time period. Individual splitting constants were obtained sequentially using a program SCAN1A (*14*). It is based upon the principle that when a correct splitting constant is found the difference characterized by the sum of the squares of the deviation between the simulated and the experimental spectrum will be minimized for the region of the spectrum from the starting point to a limit slightly larger than the chosen splitting. The major drawback of the program is that the outermost line of the spectrum needs to be located. As individual splittings are found by the SCAN1A program all previous splittings are reoptimized using the SIMPLEX procedure. In this way, fewer parameters need to be optimized in the beginning when the uncertainty of the parameters is sometimes quite large. This procedure also reduces the possibility of getting stranded at local minima which is often a problem with the SIMPLEX method when there are many variable parameters. Eventually when all splittings have been optimized the complete simulated spectrum should be similar to the experimental spectrum.

RESULTS

Benz[a]anthracene. On oxidation with TTFA/TFA a pink-colored solution was produced which gave the EPR spectrum shown in Fig. 2a. The radical was relatively stable for a few hours at temperatures below −10°C. The width of the spectrum was found to be 22.15 G but the severe overlap arising from the many nonequivalent splittings resulted in a spectrum of only moderate resolution. Close examination of the central portion of the spectrum indicated that the spectrum is not totally symmetric, suggesting perhaps that there is contamination from a second radical species. A

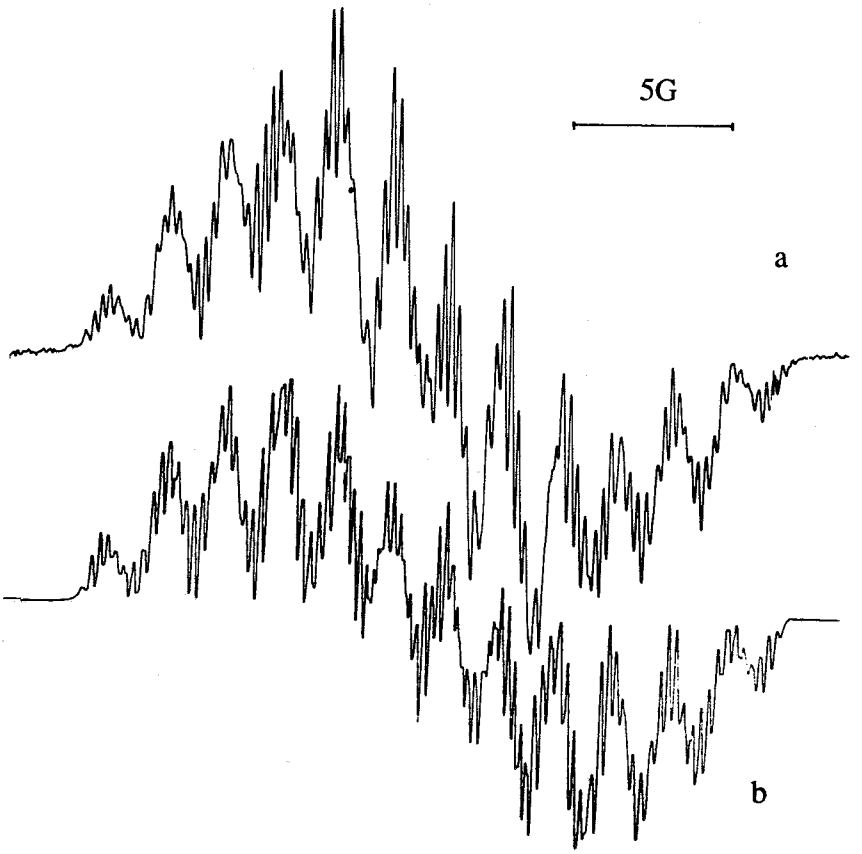

FIG. 2. (a) EPR spectra from BA in TTFA/TFA at −15°C. (b) Simulation of 7-TFA-BA‡ using splitting constants given in Table 5.

very similar spectrum was also obtained when BA was oxidized with TTFA in d-TFA (not shown) and when 7-d-BA was oxidized with TTFA/TFA. Unsuccessful attempts were made, with the cooperation of Dr. Dalal of West Virginia University, to observe an ENDOR spectrum of BA in TTFA/TFA.

When the spectrum from BA was analyzed using the numerical methods outlined under Experimental the best fit was found for the following set of splitting constants: 0.238 (three equivalent splittings), 0.334, 0.449, 0.504, 0.636, 1.304, 1.648, 2.038, 2.117, 2.915, 3.617, 5.635 G. The simulated spectrum from these splittings (Fig. 2b) is in close, but not exact, agreement with the experimental spectrum (Fig. 2a).

Methylated BAs. Oxidation of the 12 monomethylated BAs and DMBA with TTFA/TFA also resulted in pink to purple solutions which produced EPR spectra of various widths and resolutions (see Figs. 3 and 4). Well-resolved spectra whose widths could be accurately measured were obtained from 2-, 3-, 4-, 5-, 10-, 11-, and 12-ME-BAs; 7-, 8-, and 9-ME-BAs gave somewhat less resolved spectra; and 1- and 6-ME-BAs gave the most poorly resolved spectra whose widths were least accurately

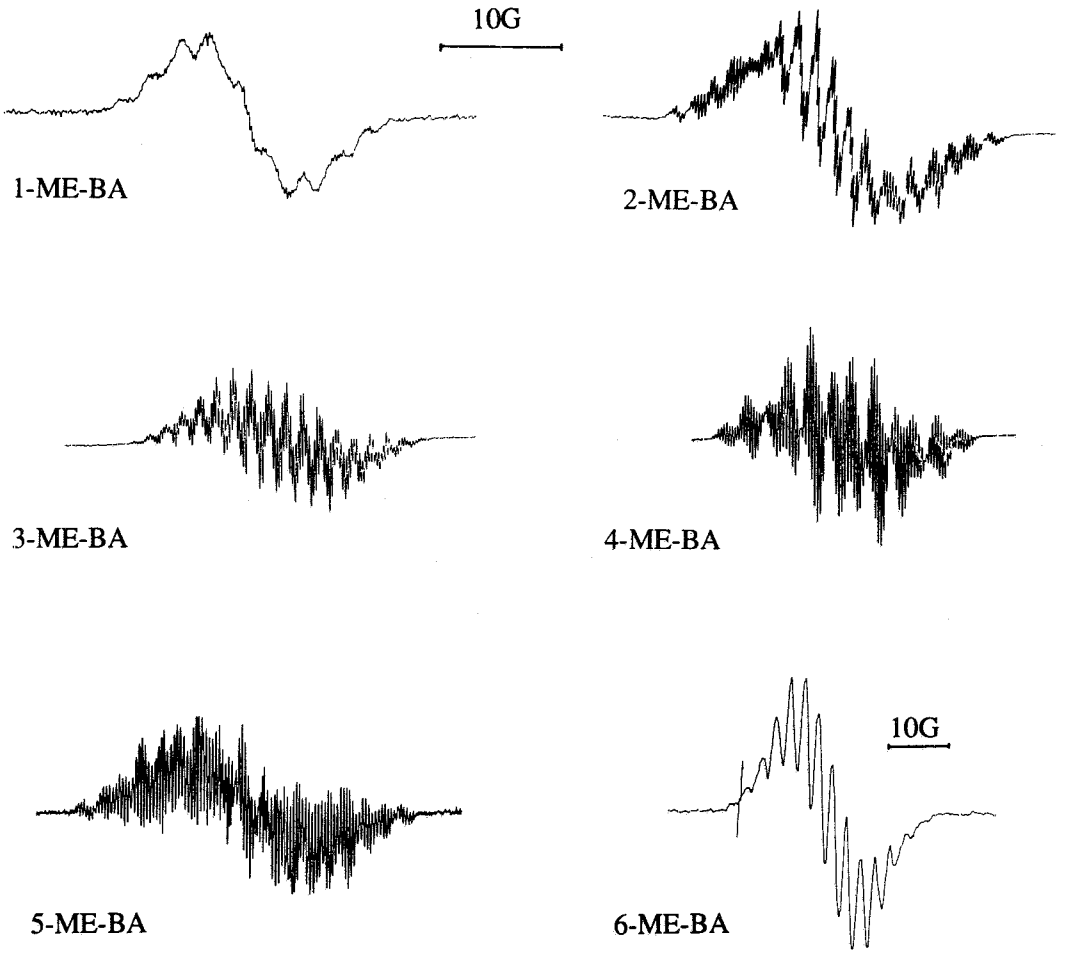

FIG. 3. EPR spectra of 1-ME-, 2-ME-, 3-ME-, 4-ME-, 5-ME-, and 6-ME-BAs in TTFA/TFA at −15°C. Note the different scale for 6-ME-BA.

measured. DMBA gave a spectrum very similar to that previously observed in H_2SO_4 (*6*). The widths of the methylated BAs are indicated in Table 1.

DISCUSSION

Radical species from BA in TTFA/TFA. There are three major reactions which have been observed when compounds are oxidized with TTFA/TFA (*15–18*). They are nuclear substitution, biaryl coupling, and side-chain substitution. The one-electron pathway for these reactions competes with a simultaneous two-electron pathway, and in the case of aromatic hydrocarbons the cation radical intermediate of the parent compound is sufficiently stable in many cases to be observed by EPR spectroscopy. This, however, is not true for anthracene which undergoes substitution and

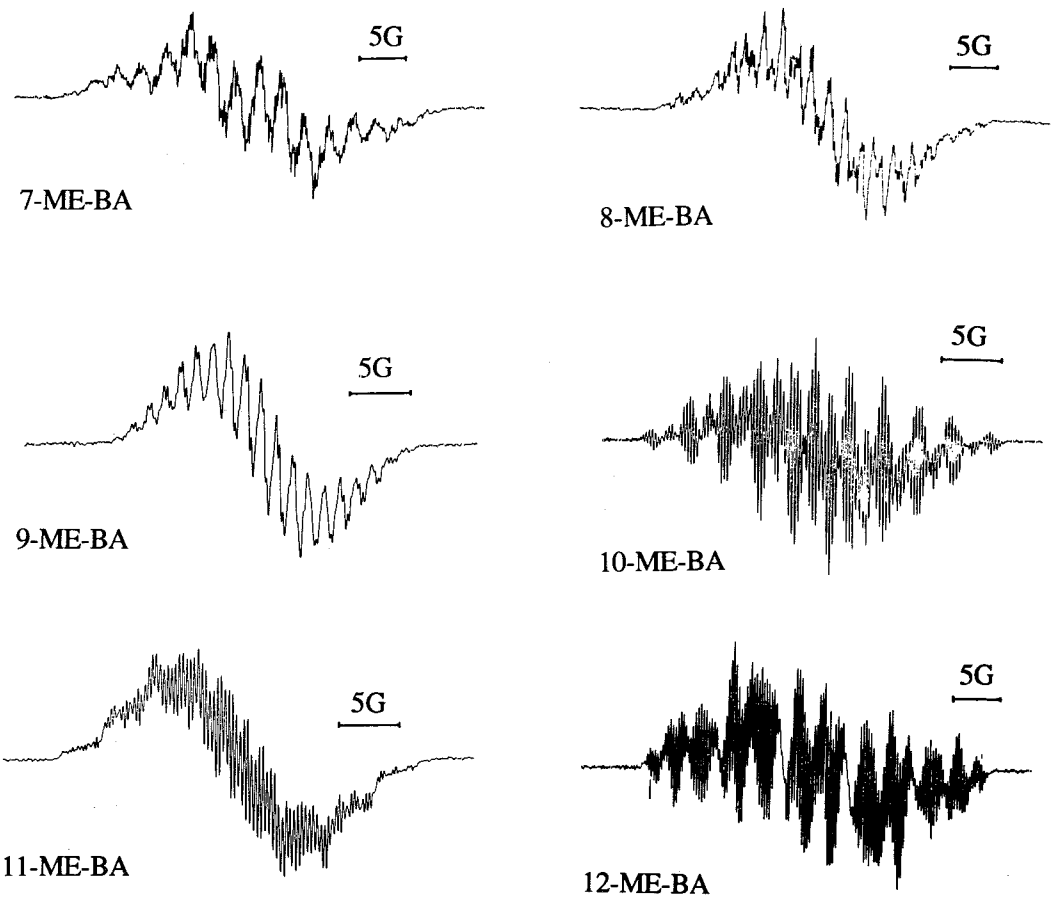

FIG. 4. EPR spectra of 7-ME-, 8-ME-, 9-ME-, 10-ME-, 11-ME-, and 12-ME-BAs in TTFA/TFA at −15°C. Note that 7-, 8-, and 12-ME-BAs are at one scale and 9-, 10-, and 11-ME-BAs are at a slightly different scale.

further oxidation at the 9 and 10 positions, resulting in the observation of EPR signals from the 9-(trifluoroacetoxy)- and 9,10-(ditrifluoroacetoxy)-anthracene cation radicals (*19*). It was also noted that when the 9 position was substituted with a methyl group, substitution was blocked at that position. It has been similarly observed that benzo[*a*]pyrene undergoes substitution at the 6 position upon oxidation with TTFA/TFA to give an EPR spectrum of the cation radical of 6-trifluoroacetoxybenzo[*a*]pyrene; on the other hand, the cation radical of 6-methylbenzo[*a*]pyrene is stable for some time on oxidation with TTFA/TFA (*9*). By analogy with these results it is possible that the radical observed from BA upon reaction with TTFA/TFA is one of four likely cation radical species, namely the BA cation radical, 7-trifluoroacetoxy-BA, 12-trifluoroacetoxy-BA, or 7,12-ditrifluoroacetoxy-BA cation radicals. The 7 and 12 positions of BA are analogous to the 9 and 10 positions of anthracene and are known to be positions of highest reactivity. Reaction products of BA in the

TABLE 1

Measured Widths of the EPR Spectra of BA and Methylated BAs in TTFA/TFA at −10°C

Methyl substitution	EPR width (gauss)	Methyl substitution	EPR width (gauss)
—	22.15 ± 0.10	7	39.28 ± 0.20
1	23.04 ± 0.05	8	34.90 ± 0.20
2	28.75 ± 0.10	9	25.30 ± 0.20
3	24.98 ± 0.10	10	30.14 ± 0.05
4	22.65 ± 0.05	11	30.06 ± 0.10
5	28.75 ± 0.10	12	36.00 ± 0.05
6	34.15 ± 0.50	7,12	56.70 ± 0.20

one-electron oxidizing systems iodine–pyridine and manganic acetate–acetic acid have indicated that the 7 position of BA is the most reactive position, closely followed by the 12 position (*20, 21*). The similarity of the EPR spectra from BA and 7-d-BA is also indicative of substitution at the 7 position. The deuterium cannot be lost by simple exchange since the exchange reaction is known to be slow (*22*) as is also shown by the similarity of the spectra from BA in TFA and d-TFA. If the deuterium were not lost by substitution the EPR spectra from BA and 7-d-BA would have been quite different due to the changed nuclear spin and splitting of the deuterium atom.

Further confirmation of the identity of the radical species from BA can be obtained by comparing the spectral widths of the radicals obtained from 7-ME-BA, 12-ME-BA, and 7,12-DMBA. If three assumptions are made, (i) that methyl substitution does not change the sum of the spin densities from all other positions, (ii) that the methyl proton splittings equal the splitting of the ring proton they replace, and (iii) the splitting of the trifluoroacetoxy group is the same whether the TFA group is attached to the 7 or 12 position, then the spectral widths can be expressed as the sum and differences of four components. These are the width of the BA cation radical, B, which equals the sum of all 12 proton splittings, the splitting of the protons at the 7 (H_7) and 12 (H_{12}) positions, and the splitting of a TFA group (T). The most likely species formed and the corresponding equations for each possibility are listed in Table 2. For BA only the 7-substituted and 7,12-disubstituted derivatives are considered because of the evidence for 7 substitution cited above. For the 7- and 12-ME-BAs only monosubstitution or no TFA substitution is possible, and for DMBA no TFA substitution is possible. Eight possible sets of simultaneous equations can be formed by choosing one species for each compound. The solutions to these equations are shown in Table 3. Set 4, comprising 7-TFA-BA$^+$, DMBA$^+$, 7-ME-12-TFA-BA$^+$, and 12-ME-7-TFA-BA$^+$, gave the most reasonable solution, since all other sets gave large values for the TFA group splitting, which by analogy to anthracene and benzo[a]pyrene is expected to be quite small.

The simulation of the EPR spectrum of BA in TTFA/TFA is also consistent with the formation of 7-TFA-BA$^+$ since the group of three equivalent splittings of 0.238 G, presumably from the three fluorines of the trifluoroacetoxy group, is similar to the

TABLE 2

Possible Species Formed and Equations for Total Width of EPR Spectra

Reactant in TTFA/TFA	Possible species	Equation for width
BA	1a 7-TFA-BA$^+$	$B - H_7 + T = 22.15$
	1b 7,12-TFA-BA$^+$	$B - H_7 - H_{12} + 2T = 22.15$
DMBA	2a DMBA$^+$	$B + 2H_7 + 2H_{12} = 56.70$
7-ME-BA	3a 7-ME-BA$^+$	$B + 2H_7 = 39.28$
	3b 7-ME-12-TFA-BA$^+$	$B + 2H_7 - H_{12} + T = 39.28$
12-ME-BA	4a 12-ME-BA$^+$	$B + 2H_{12} = 36.0$
	4b 12-ME-7-TFA-BA$^+$	$B + 2H_{12} - H_7 + T = 36.0$

0.276 splitting found for 9-trifluoroacetoxy anthracene (*19*). The sum of the three fluorine splittings in the TFA group (0.714 G) is somewhat less than the 3.37 G calculated in Table 3, but the assumptions used to set up the equations in Table 2 should be considered approximate. In addition to the three equivalent splittings, 11 nonequivalent proton splittings were found as expected for 7-TFA-BA$^{+\cdot}$. As pointed out above the agreement between the simulated and the experimental spectra is not perfect. However, the slight asymmetry of the experimental spectrum suggests that it may contain a contribution from a second radical species which could be 7,12-DiTFA-BA$^{+\cdot}$.

Radical species from monomethyl-BAs in TTFA/TFA and estimated methyl splittings. It has been shown previously that the effect of methyl substitution on the radical products from benzo[*a*]pyrene is negligible in most cases unless the substituent blocks a reactive position (*10*). It is to be anticipated that methyl substitution in BA would have little effect on the formation of the 7-trifluoroacetoxy cation radical unless it occurs at or near the 7 position. For those ME-BAs whose products from one-electron oxidation reactions have been investigated (*20*) it was found that 2-, 5-, 11-, and 12-ME-BAs gave exclusively substitution at the 7 position. 6- and 8-ME-BA gave

TABLE 3

Solutions of the Simultaneous Equations Formed from the Data in Table 2

Possible species	B	H_7	H_{12}	T
(1) 1a, 2a, 3a, 4a	18.58	10.35	8.71	13.97
(2) 1a, 2a, 3a, 4b		No solution		
(3) 1a, 2a, 3b, 4a	8.16	10.35	13.92	24.34
(4) 1a, 2a, 3b, 4b	26.80	8.02	6.93	3.37
(5) 1b, 2a, 3a, 4a	18.58	10.35	8.71	11.32
(6) 1b, 2a, 3a, 4b	17.30	10.99	8.71	12.27
(7) 1b, 2a, 3b, 4a	15.10	10.35	10.45	13.93
(8) 1b, 2a, 3b, 4b		No solution		

mixtures of 7- and 12-substituted products and 7-ME-BA gave predominantly a 12-substituted product. Unfortunately, in our studies, due to the small amounts of materials available, it was not possible to investigate in any detail the identity of the products formed in each case. As was indicated in the previous section the measured widths of the radicals from 7- and 12-ME-BAs are consistent with the formation of 12-TFA-7-ME-BA^{+} and 7-TFA-12-ME-BA^{+}, respectively. If it is assumed that the radicals observed are 7-TFA-substituted BAs except for 6-, 7-, and 8-ME-BAs it is possible to estimate the methyl splittings in each case from the measured spectral widths. Thus the increase in spectral width of a ME-BA over that of BA in TTFA/TFA is equal to twice the methyl proton splitting, assuming that methyl substitution does not change the sum of the splittings from all the other unsubstituted positions. The estimated splitting can be calculated assuming either 7- or 12-TFA substitution. In the latter case, the width of the 12-TFA-BA cation radical should be subtracted from the measured width. Since 12-TFA-BA^{+} has not been observed its width must be calculated from the data in Table 2. Similarly, for 7-ME-BA, the width of 12-TFA-BA^{+} must be used to give the estimated methyl splitting. The estimated methyl proton splittings obtained in this way are shown in Table 4.

Assignment of splittings in 7-TFA-BA^{+}. The estimated methyl splittings derived above can be used to suggest an assignment for the splittings obtained for the 7-TFA-BA cation radical. If it is assumed that methyl substitution does not perturb the spin density distribution to a large extent, then the order of estimated methyl splittings should reflect the order of proton splittings in the unmethylated compound. The magnitude of the proton splittings is usually found to be somewhat less than the estimated methyl splittings, especially at the high spin density positions. This is due,

TABLE 4

Estimation of Methyl Proton Splittings from Spectral Widths

Methyl position	Spectral width (gauss)	Anticipated TFA substitution	Estimated CH_3 splittings[a] (gauss)
1	23.04 ± 0.50	7	0.15–0.73
2	28.75 ± 0.10	7	3.2–3.4
3	24.98 ± 0.10	7	1.31–1.51
4	22.65 ± 0.05	7	0.15–0.35
5	28.75 ± 0.10	7	3.2–3.4
6	34.15 ± 0.50	7 and/or 12	5.7–6.3 or 5.2–5.8[b]
7	39.28 ± 0.20	12	7.9–8.1
8	34.90 ± 0.20	7 and/or 12	6.2–6.5 or 5.7–6.0[b]
9	25.30 ± 0.20	7	1.42–1.72
10	30.14 ± 0.05	7	3.92–4.07
11	30.06 ± 0.10	7	3.85–4.05
12	36.00 ± 0.05	7	6.85–6.9

[a] Estimated CH_3 splitting for 7-TFA substitution = (spectral width − 22.15)/2, since width of 7-TFA-BA^{+} is 22.15 G.

[b] Estimated CH_3 splitting for 12-TFA substitution = (spectral width − 23.2)/2, where estimated width of 12-TFA-BA^{+} = $B - H_{12} + T$ (from Table 3).

no doubt, to the charge dependence of methyl group splittings (*23*). From the estimated splittings in Table 4, one would predict that the order of proton splittings should be $7 > 12 > 8 > 6 > 10 \approx 11 > 5 \approx 2 > 9 \approx 3 > 4 \approx 1$. The assignment of splittings for 7-TFA-BA‡ based on this order is shown in Table 5. A comparison with the splittings calculated for BA‡ from a simple McLachlan modified HMO calculation (*6*) shows general agreement with the values proposed.

Correlation with biological activity. It is well known that 7- and 12-ME-BAs are potent and moderately potent carcinogens and mutagens (*24, 25*) and that substitution at both 7 and 12 positions gives the very potent DMBA. It is also found that 6- and 8-ME-BA are also moderately potent carcinogens and mutagens whereas the other methyl substituted BAs are found to be weak or inactive carcinogens (*26*). No clear explanation has yet been given for the increased mutagenicity and carcinogenicity resulting from methyl substitution at the 6, 7, 8, or 12 positions. It is, however, apparent from our results that these are, in fact, the positions of highest spin, and hence electron density, in the highest occupied molecular orbital of BA. The effect of methyl substitution on the metabolism of BA to ultimately carcinogenic forms could occur through the formation of hydroxymethyl derivatives. The *in vivo* formation of hydroxymethyl derivatives could proceed through an enzymatic one-electron oxidation to form an intermediate cation radical which would be susceptible to nucleophilic attack at positions of high electron density. If methyl groups are substituted at such positions, loss of a methyl proton can yield a benzylic carbonium ion which can react with water to give the hydroxymethyl derivative. The formation of hydroxymethyl derivatives may serve to activate the hydrocarbon in one of two ways. The hydroxymethyl derivative may undergo further reaction to produce hydroxymethyl esters which are often highly mutagenic compounds which can bind to DNA *in vitro* (*27–29*). Alternatively the hydroxymethyl derivatives may have an increased affinity

TABLE 5

Assignment of Splitting Constants in 7-TFA-BA‡ and Comparison with HMO Calculation for BA‡

Position	Splitting assignment 1	HMO calculated splittings[a]
1	0.33	0.59
2	1.30	0.88
3	0.50	0.16
4	0.45	0.52
5	1.65	2.15
6	2.91	1.95
7	0.238 (3F)	6.91
8	3.61	3.18
9	0.64	0.72
10	2.12	1.21
11	2.04	2.76
12	5.63	5.71

[a] $Q = 25$ G.

for the metabolizing enzymes over that of the parent compound (*30*). Such increased affinity might lead to the preferential metabolism of the hydroxymethyl derivatives to ultimately carcinogenic diol–epoxide derivatives.

There is considerable information on the metabolism of 6-, 7-, 8-, and 12-ME-BAs (*31–33*) and it is interesting to note that hydroxymethyl metabolites are found in significant amounts. A study of the formation and reactivity of hydroxymethyl derivatives from all 12 monomethylated BAs might provide more detailed information to correlate with the electron density predictions.

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